A Possible Bioherbicide for *Avena fatua* L. (Wild Oats): Isolate Collection and Host Range Testing

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A survey of the diseases of wild oats was carried out in NSW during 1995 and 1996. This survey identified a variety of fungal pathogens. Because of its virulence in laboratory tests and its impact in the field, *Drechslera avenacea* was chosen as the basis of a potential bioherbicide against *Avena fatua*. A variety of cultivars of wheat, barley and oats were inoculated with 12 diverse isolates of this fungus and the disease reaction assessed in each case. In general, *Avena fatua* was most susceptible to the pathogen, followed in order of susceptibility by oats, barley and wheat. An isolate's suitability for incorporation into a bioherbicide was based upon the severity of the disease interaction (measured as percentage leaf necrosis ten days after inoculation). Isolates with good potential caused severe symptoms on *A. fatua* and were less virulent on cereal cultivars. Of the tested isolates, two provided good selectivity and were chosen for further study. As a result of the large number of stored isolates it is possible that other selective isolates may be found.

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INTRODUCTION

Wild oat, *Avena fatua* L. is a serious weed of a wide range of crops. These include both dicotyledons (e.g. peas, beans, potatoes, sunflower) and cereal crops (Holm et al. 1977). In Australia, wild oats are principal weeds of cereals including wheat, barley and oats. Its effect on wheat is through competition for light (Rooney 1991), nutrients (Kirkland 1993) and also through allelopathy (Perez and Ormeno-Nunez 1991). Herbicide resistant populations of *Avena* spp (Powles and Holtum 1992) have been recorded in Australia. Hence, calls for augmentation of chemical controls and the diversification of control strategies have been made (Howat 1987).

The advantages of biological control in reducing use of chemicals, non-target damage and environmental contamination are well known. Application of pathogenic organisms to grass weeds remains a scarcely investigated option for weed control (Evans 1991). Several attempts have been made to develop bioherbicides effective against wild oats. In Canada, a bioherbicide was developed based on the fungal pathogens *Phytophthora palmivora* and *Colletotrichum gloeosporioides* f.sp. aeschymene (*sic.*) (Mortensen 1983). *Pyrenophora semeniperda*, a fungal pathogen capable of killing seed, can reduce germination, emergence and vigour of wild oats and is also the subject of biological control research (Medd and Campbell 1996).

Recently attention has been given to *Drechslera avenacea* (Curtis and Cooke) Shoem (teleomorph: *Pyrenophora chaetomioides*). Wilson (1987) found that *D. avenae* (Eidam) Scharif [a synonym of *D. avenacea*; (P.M. Kirk, in litt.)] reduced seedling root and shoot growth by 15% while not affecting winter wheat. However, in this case, the

competitive ability of the weed was unaffected. In China, isolates of *D. avenacea* have been collected from naturally infected wild oats in wheat fields, cultured and pathogenicity tested. The fungus infected wild oats but not wheat (Zonjian and Yanghan 1996).

A. fatua is widely distributed across a range of environments and climates; additionally variable phenotypes have been recorded (Whalley and Burfitt 1972). Survey and collection of pathogenic fungi across the geographic and physical ranges of the weed will potentially yield fungal isolates which will be useful as the principal component of a bioherbicide. *A. fatua* is also closely related to the cereal crops, notably cultivated oats (*A. sativa* L). The susceptibility of these crops to the biological control agent must be assessed in the context of wheat/pasture rotations.

Our objective in this study was to find suitable endemic fungi as the basis of a bioherbicide. We made no assumptions as to the suitability of candidates before the project, preferring to base our judgement on preliminary pathogenicity tests of all fungi collected. Based on the severity of symptoms observed in the field and following artificial inoculation, *D. avenacea* was chosen as the most likely candidate for development as a bioherbicide.

MATERIALS AND METHODS

Survey of the diseases of wild oats in New South Wales

Diseased wild oats (*Avena* spp) were collected from the field during 1995 and 1996. Plants were collected from roadsides, cultivation (principally wheat) and fallow paddocks. Survey dates were chosen so that plants of various ages could be collected. The area chosen for surveys was based largely on the NSW wheat belt bounded in the north-west by Bourke, the north-east by Glen Innes, the south-west by Hay and Deniliquin and the south-east by Cootamundra and Wagga Wagga. The survey also included several peripheral areas including the Grafton area and semi-rural areas west of Sydney.

At each site whole diseased plants were collected and stored at 4°C until they were returned to the laboratory. Where possible, the collection included an inflorescence to confirm host identification; where seedlings were collected this was not possible.

Lesions from diseased plants were photographed, assigned a code number, excised and cut into three portions before isolations took place. The portions were soaked in 2% sodium hypochlorite for one, two or three minutes, dried on a sterile absorbent paper and plated onto quarter strength Potato Dextrose Agar (Merck, Darmstadt, Germany), supplemented with 2ml/l of 25% lactic acid to retard bacterial growth. Where fungi grew from lesions after five days, the fungus was subcultured onto half strength V8 (Campbells Soups Australia) agar and placed in a dark cabinet at 20°C until sporulation was observed.

A series of limited host range tests on *Avena fatua* and wheat (cv. Dollarbird) were conducted for representative isolates of all genera collected.

Host range testing of Drechslera avenacea

The pathogenicity of twelve isolates identified as *D. avenacea* by the International Mycological Institute (IMI, Kew, UK) was tested against 6 cultivars of wheat, 2 cultivars of barley, 4 cultivars of oats and wild oats. The isolates chosen had geographically diverse origins. The cultivars tested were resistant to a range of diseases (Gammie 1995) (Table 1).

A conidial suspension (1×10^5 conidia per ml) was made for each isolate. This was applied to 5 seedlings of all of the chosen cultivars. The seedlings were at approximately the three leaf stage at the time of inoculation. Inoculated seedlings were placed in

TABLE 1

The cereal cultivars tested for their susceptibility to *D. avenacea*. The cultivars were chosen to represent variation in their response to a number of fungal diseases.

		Disease susceptiblity[1]	
Cereal	Cultivar	Susceptible	Resistant
Oats	Bimbil	leaf rust stem rust	BYDV[2] (moderately)
	Echidna	leaf rust stem rust	BYDV[2] (moderately)
	Mortlock	leaf rust stem rust	BYDV[2] (moderately)
	Coolabah	leaf rust stem rust	BYDV[2] (moderately)
Barley	O'Connor	net blotch (moderately) powdery mildew (very)	leaf scald (moderately) covered smut
	Skiff	leaf scald powdery mildew (moderately)	net blotch (moderately) covered smut
Wheat	Dollarbird	*Septoria tritici* blotch yellow spot	flag smut leaf rust stem rust
	Rosella	flag smut leaf rust stem rust *Septoria tritici* blotch yellow spot	
	Hartog	crown rot *Septoria tritici* blotch yellow spot	flag smut leaf rust stem rust
	Janz	crown rot *Septoria tritici* blotch (moderately) yellow spot	flag smut leaf rust stem rust
	Sunbrook	yellow spot	flag smut leaf rust stem rust *Septoria tritici* blotch (moderately)
	Swift	yellow spot	flag smut leaf rust stem rust Septoria tritici blotch (moderately)

1. According to Gammie 1995

2. BYDV = Barley Yellow Dwarf Virus

a dew chamber (Percival, Iowa, USA) at 20°C for 16 hours, so that free moisture formed on the leaf surface. They were then placed in a controlled environment cabinet at 20°C with a 12 hr light/dark cycle. After ten days the percentage of necrotic tissue on the two oldest leaves was assessed visually. The average value for the five plants per treatment was used to generate an index of plant susceptibility. An isolate's suitability as the active ingredient in a bioherbicide was based upon its fulfilment of the following criteria:

1. Index category 8 (more than 90% of leaf tissue necrotic) on wild oats
2. Index category 0, 1 or 2 (0–4% of leaf tissue necrotic) on wheat
3. Index category 0, 1 or 2 (0–4% of leaf tissue necrotic) on barley
4. Index category 5 (65% of leaf tissue necrotic) or lower on oats.

RESULTS

Survey of the diseases of wild oats in New South Wales

One thousand two hundred and twelve fungal isolates were obtained during the survey. These isolates include *Drechslera avenacea*, *Stagonospora avenae*, *Leptosphaerulina trifolii*, *Pleospora infectoria*, *Stemphylium vesicarium*, *Colletotrichum sublineolum*, *Bipolaris australiensis*, *Phoma subglomerata*, *Pyrenophora semeniperda*, *Epicoccum purpurascens*, *Curvularia* sp. and *Ascochyta agropyrina*.

Koch's postulates were applied and confirmed that *D. avenacea*, *S. avenae*, *L. trifolii*, *P. infectoria*, *P. subglomerata* and *P. semeniperda* infect wild oats but had little or no effect on wheat. *C. sublineolum* and *Curvularia* sp. infected both wheat and wild oats.

Drechslera avenacea was chosen for closer study because of the foliar damage caused by this pathogen in artificial inoculations and its observed impact in the field. It was collected at a large number of diverse sites (Fig. 1). Some 400 isolates of this species have been lyophilised and stored.

Host range testing of Drechslera avenacea

The susceptibility of cultivars varied according to the identity of the challenging isolate (Table 2). No isolate fulfilled the desirable criteria perfectly. The best performing isolates were IMI374564 which caused a more severe disease reaction on all cultivars of oats than was desirable and IMI375958 which caused a more severe disease reaction on the oat cultivar Bimbil than was desirable.

The majority of isolates (eight of twelve) caused severe disease on all cultivars of cultivated oats. No isolate caused severe disease on wheat or barley.

DISCUSSION

Genetic diversity of pathogenic fungi may be exploited in order to obtain the best isolates for use as the basis of bioherbicides. Isolates should cause severe damage to their target weed and be selective, in that they do not infect other plants; particularly valuable, closely related crop species. Widely distributed, distinct populations of fungi of the same species may demonstrate genetic variation best suiting them to particular habitats. A logical preliminary to examining potential bioherbicide fungi was to investigate this variation through extensive surveys.

While a large number of fungal species were isolated over the two year period of this study, *Drechslera avenacea* is the most likely to be used in a bioherbicide. The

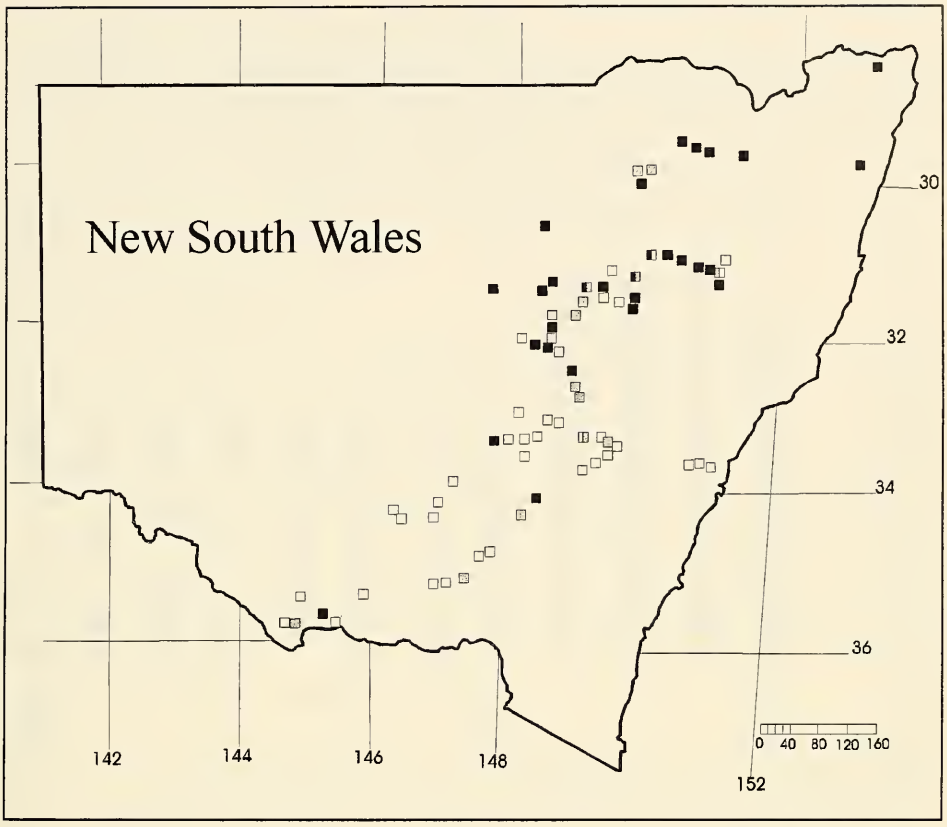

Figure 1. Survey sites from which isolates of *Drechslera avenacea* were collected during 1995 and 1996. Fungi were isolated from *Avena fatua* or *A. ludoviciana* (❑) and *A. barbata* (■). In other cases, (▩) it was not possible to determine the host identity to specific level.

severity of disease it causes, its wide distribution and selectivity mean that it is potentially robust and effective. The isolation of fungi from infected plants in hot, dry areas (e.g. Deniliquin: 300–400 mm median annual rainfall) indicates that this isolate is either specifically adapted to these conditions or the species as a whole can tolerate a wide range of environmental conditions. Given this observation, certain isolates of this fungus may require only a short dew period; a desirable characteristic for a fungus which is to be the basis of a bioherbicide. Further testing remains to be done in this area. Should these speculations prove true the large number of isolates collected and stored during this study will enable us to further refine the bioherbicide through a more intensive study of other isolates. Isolates have also been collected from southern Queensland, Victoria and Western Australia.

The susceptibility of cultivated oats to isolates tested is a concern. The fungus causes eye spots, pre-emergence and post-emergence seedling blight, leaf stripes of young plants and leaf spots of mature oats (Sivanesan 1987); crop losses of 2–10% have been reported in Finland (Rekola et al. 1970). However, two points should be considered. Firstly, this study identified a great deal of variation in the virulence of isolates; for example, for the cultivar Echidna, the area of necrotic leaf tissue as a result of inoculation varied from 7% to 97.2% dependent upon which isolate was applied. Careful screening of

TABLE 2

Susceptibility1 of a range of cereal cultivars to twelve isolates of *Drechslera avenacea* which were collected from regions throughout New South Wales. Highlighted numbers indicate interactions which fulfilled the criteria desirable for the isolate to be used as a bioherbicide outlined in the text.

	Isolate accession number											
Cultivar	IMI375958	IMI375692	IMI375961	IMI375959	IMI374564	IMI374567	IMI374568	IMI374565	IMI375957	IMI374572	IMI374566	IMI374570
Bimbil	6	**4**	**4**	**4**	8	6	6	6	8	8	6	8
Echidna	**5**	**3**	**4**	**4**	7	7	7	7	8	7	7	8
Mortlock	**5**	**3**	**3**	**3**	8	6	6	6	6	6	8	8
Coolabah	**5**	**4**	**4**	**5**	8	7	7	8	8	7	7	8
O'Connor	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**2**
Skiff	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	3
Dollarbird	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**2**	4	**1**	3	3
Rosella	**1**	**1**	**0**	**1**	**2**	**1**	**2**	**1**	**1**	4	**2**	4
Hartog	**1**	**1**	**0**	**0**	**1**	**1**	**1**	**1**	3	**2**	3	3
Janz	**1**	**1**	**0**	**1**	**1**	**1**	**2**	**1**	**1**	**2**	**2**	3
Sunbrook	**1**	**1**	**1**	**1**	**1**	**1**	**0**	3	**1**	**1**	**1**	**0**
Swift	**1**	x	x	x	**2**	**2**	**2**	**1**	**1**	3	3	3
Wild Oats	**8**	4	4	6	**8**	7	5	6	6	7	6	**8**

[1] 0: 0% of leaf area necrotic; 1: 0–2% of leaf area necrotic; 2: 2–4% of leaf area necrotic; 3: 4–9% of leaf area necrotic; 4: 9–50% of leaf area necrotic; 5: 50–65% of leaf area necrotic; 6: 65–80% of leaf area necrotic; 7: 80–90% of leaf area necrotic; 8: 90–100% of leaf area necrotic; x: interaction not assessed.

isolates will maximise selectivity. Secondly, while dispersal mechanisms for *D. avenacea* have not been studied, it may be assumed that they are typical of the "Helminthosporium" group in which conidia are dispersed only a short distance by rain splash, wind-driven rain, or air currents. Because spores are relatively large, dispersal is only over short distances (Agrios 1997). Applications of *D. avenacea* as a bioherbicide in wheat cultivation are unlikely to spread to nearby oat cultivation.

Through the protocol adopted in this study a number of isolates have been selected for further study. Studies of disease etiology remain to be done.

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